

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>C07K 7/18, A61K 38/08</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 97/09346</b> <b>(43) International Publication Date:</b> 13 March 1997 (13.03.97)
<b>(21) International Application Number:</b> PCT/US96/13614 <b>(22) International Filing Date:</b> 22 August 1996 (22.08.96) <b>(30) Priority Data:</b> 08/526,764                      8 September 1995 (08.09.95)                      US <b>(71) Applicants:</b> CORTECH, INC. [US/US]; 6850 North Broadway, Denver, CO 80221 (US). UNIVERSITY TECHNOLOGY CORPORATION [US/US]; Suite 250, 3101 Iris Avenue, Boulder, CO 80301 (US). <b>(72) Inventors:</b> WHALLEY, Eric, T.; 15955 West 77th Place, Golden, CO 80403 (US). STEWART, John, M.; 3690 East Dartmouth Avenue, Denver, CO 80210 (US). GERA, Lajos; 880 Cherry Street #208, Denver, CO 80220 (US). <b>(74) Agent:</b> BIRD, Donald, J.; Pillsbury, Madison & Sutro, Cushman, Darby & Cushman Intellectual Property Group, 9th floor - 1100 New York Avenue N.W., Washington, DC 20005-3918 (US).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> DES-ARG <sup>9</sup> -BK ANTAGONISTS  <b>(57) Abstract</b>  The present invention provides des-Arg <sup>9</sup> bradykinin analogs which are selective B1 receptor antagonists.		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

## DES-ARG9-BK ANTAGONISTS

### FIELD OF THE INVENTION

The invention relates to novel biologically active peptides which act as  
5 antagonists of the biological activities of bradykinin (BK) and related kinins, in particular, desArg<sup>9</sup>-BK and desArg<sup>10</sup> kallidin, the pharmaceutically acceptable salts of these antagonists, and their use as therapeutic agents.

### BACKGROUND OF THE INVENTION

10 Bradykinin (BK), a nonapeptide (Arg<sup>1</sup>-Pro<sup>2</sup>-Pro<sup>3</sup>-Gly<sup>4</sup>-Phe<sup>5</sup>-Ser<sup>6</sup>-Pro<sup>7</sup>-Phe<sup>8</sup>-Arg<sup>9</sup>) and its physiologically important related peptides, kallidin (Lys-bradykinin) and Met-Lys-bradykinin, exhibit physiological actions which qualify them as mediators of inflammatory reactions, hypotensive states, and pain. Bradykinin is overproduced in pathological conditions such as septic shock  
15 (Robinson et al., *Am. J. Med.* 59: 61 (1975)) and hemorrhagic shock (Hirsch et al., *J. Surg. Res.* 17: 147 (1974)), anaphylaxis (Collier and James, *J. Physiol.* 160: 15P (1966)), arthritis (Jasani et al., *Ann. Rheum. Dis.* 28: 497 (1969); Hamberg et al., *Agents Actions* 8: 50 (1978); Sharma et al., *Arch. Int. Pharmacodyn.* 262: 279 (1983)), rhinitis (Proud et al., *J. Clin. Invest.* 72: 1678  
20 (1983); Naclerio et al., *Clin. Res.* 33: 613A (1985)), asthma (Christiansen et al., *J. Clin. Invest.* 79: 188 (1987)), inflammatory bowel disease (Zeitlin and Smith, *Gut* 14: 133 (1973)), and certain other conditions including acute pancreatitis, post-gastrectomy dumping syndrome, carcinoid syndrome, migraine, and hereditary angioedema (Leme, *Handb. Exp. Pharmacol.* 50/I: 464 (1978)). The  
25 production of bradykinin results in pain at the site of the pathological condition, and the overproduction intensifies the pain directly or via stimulation by bradykinin of the activation of the arachidonic acid pathway which produces prostaglandins and leukotrienes, more distal mediators of inflammation (Handbook of Experimental Pharmacology, Vol. 25, Springer-Verlag (1969),  
30 and Vol. 25 Supplement (1979); Stewart, in "Mediators of the Inflammatory Process," Henson and Murphy, eds., Elsevier, (1989)).

Bradykinin has been found to be produced in inflammatory reactions in the intestine, provoking contraction of smooth muscle and secretion of fluid and ions. The existence of specific bradykinin receptors in the mucosal lining of the intestine and in intestinal smooth muscle is demonstrated by Manning et al.

5 (Nature 229: 256 (1982)), showing the influence of bradykinin in very low concentrations upon fluid and ion secretion.

The production of bradykinin and associated pain in angina has been studied and reported (Kimura et al., *Amer. Heart J.* 85: 635 (1973); Staszewska-Barczak et al., *Cardiovasc. Res.* 10: 314 (1976)). The reported action of  
10 bradykinin and prostaglandins acting in concert are the natural stimulus for excitation of the sensory receptors signalling the pain of myocardial ischemia.

Bradykinin in combination with interleukin-1 has been shown to stimulate angiogenesis *in vivo* (*Br.J.Pharmacol.* 109:14-17 (1993)).

Bradykinin and bradykinin-related kinins are not only produced  
15 endogenously, but may also be injected into an animal via stings or bites. It is known that insects such as hornets and wasps inject bradykinin related peptides that cause pain, swelling and inflammation.

Bradykinin and related peptides exert their actions on biological systems by combining with specific receptors on cell membranes in the affected tissues.  
20 These receptors are of two classes, designated B1 and B2. The B2 receptors require the entire bradykinin sequence for effective receptor combination and production of the biological effects, whereas the B1 receptors do not respond to intact bradykinin, but respond selectively to bradykinin lacking the carboxy-terminal arginine residue; designated [des-Arg<sup>9</sup>]-bradykinin. [des-Arg<sup>9</sup>]-  
25 bradykinin is produced in the body by one of the enzymes that normally destroys bradykinin, the plasma enzyme carboxypeptidase N, which removes the carboxy-terminal arginine residue. Essentially all normal physiological responses and many pathophysiological responses to bradykinin are mediated by B2 receptors, whereas in certain damaged tissues and in certain kinds of chronic inflammation,  
30 B1 receptors are induced.

The search for understanding of the mechanisms of action of bradykinin, which is essential for the development of useful tools for diagnostic use, and for the development of therapeutic agents aimed at alleviating the intense pain and other symptoms caused by the overproduction of bradykinin, was severely  
5 hindered by the lack of specific sequence-related competitive antagonists of bradykinin until the discovery of the first effective bradykinin antagonists by Vavrek and Stewart in 1985 (Vavrek et al., *Peptides* 6:161-164 (1985); U. S. Patent 4,693,993). In these early antagonists, the proline residue at position 7 of  
10 bradykinin was replaced by a D-aromatic amino acid residue, usually D-phenylalanine or D-thienylalanine. Subsequently, many modifications of the original bradykinin antagonists have been described (reviewed by J. M. Stewart and R. J. Vavrek in R. M. Burch, ed., "Bradykinin Antagonists," Pergamon, 1990), but most effective antagonists have had an aromatic amino acid residue at positions 5 and 8 and a D-aromatic residue at position 7. In certain antagonists,  
15 positions 5, 7, and 8 are occupied by aliphatic amino acid residues (J. M. Stewart, et al. in "Peptides 1992," C.H. Schneider and A.N. Eberle, eds., ESCOM, Leiden, 1993, pp 691-692).

Several peptide antagonists of BK exhibit selectivity for the B1 receptor. The basic manipulation for obtaining a selective and competitive antagonist for  
20 kinins on the B1 receptor systems is to replace the aromatic residue Phe<sup>8</sup> in desArg<sup>9</sup>-BK (or Phe<sup>9</sup> in desArg<sup>10</sup>-kallidin (KD)) by an amino acid possessing an aliphatic side chain, Leu being optimal. Thus, [Leu<sup>8</sup>, des-Arg<sup>9</sup>]-bradykinin and Lys-[Leu<sup>8</sup>, des-Arg<sup>9</sup>]-bradykinin are effective B1 receptor antagonists. However, Ala, Ile, cyclohexylAla, norLeu and D-Leu substitutions also yield B1  
25 antagonists of lower affinity.

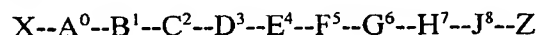
[Leu<sup>8</sup>, des-Arg<sup>9</sup>]-bradykinin has been shown to inhibit the angiogenic response elicited by bradykinin (Hu et al., *Br.J.Pharmacol.* 109:14-17 (1993)). In contrast, B2 receptor antagonists failed to inhibit this response. Thus, B1 antagonists may prove effective against angiogenic diseases such as rheumatoid  
30 arthritis, diabetic retinopathy and cancer. [Leu<sup>8</sup>, des-Arg<sup>9</sup>]-bradykinin has also been shown to cause a reduction in type I diabetic hyperglycemia (Zuccollo et

al., Proceedings of the Fourteenth International Symposium on Kinins (1995)).  
Again, B2 receptor antagonists failed to have an effect on hyperglycemia.

However, these B1 antagonists' effectiveness is hindered by their lack of stability and short term of action. Thus, a need exists for potent, stable  
5 compounds capable of selectively antagonizing the B1 receptor with long duration of action.

### SUMMARY OF THE INVENTION

The present invention provides des-Arg<sup>9</sup> bradykinin antagonists (BKA)  
10 of the general formula:



wherein

X is an aromatic, aliphatic, aromatic-aliphatic, alicyclic, heterocyclic or urethane-type acylating group, or at least one amino acid;

15 A<sup>0</sup>, B<sup>1</sup>, C<sup>2</sup>, D<sup>3</sup>, and E<sup>4</sup> are basic or neutral aromatic, aliphatic, heterocyclic, or alicyclic amino acids or A<sup>0</sup> is absent;

G<sup>6</sup> is an aromatic, aliphatic, heterocyclic, or alicyclic amino acid;

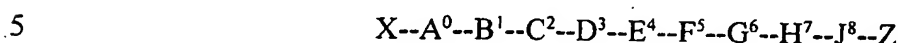
F<sup>5</sup>, H<sup>7</sup> and J<sup>8</sup> are aromatic, aliphatic, aliphatic heterocyclic, or alicyclic amino acids, provided that at least one of F<sup>5</sup>, H<sup>7</sup> and J<sup>8</sup> is selected from  
20 cyclopentane-, cyclohexane- or indane-substituted glycine; and  
Z is COOH.

### DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a new class of des-Arg<sup>9</sup> bradykinin  
25 analogs which are highly potent and selective B1 receptor antagonists with longer duration of action and greater stability than prior des-Arg<sup>9</sup> derivatives.  
The present invention provides modified bradykinin antagonist peptides that contain amino acids, preferably glycine, substituted on the  $\alpha$ -carbon or on the  $\alpha$ -nitrogen by 1-indanyl or 2-indanyl groups, or cyclopentyl or cyclohexyl groups.  
30 In a preferred embodiment, the bradykinin antagonist peptides of the present

invention contain substituted amino acid residues at positions five, seven and eight of the bradykinin native sequence.

The bradykinin antagonist peptides of the present invention may be characterized as follows:



wherein

X is the amino-terminal amino group or an aromatic, aliphatic, aromatic-aliphatic, alicyclic, heterocyclic or urethane-type acylating group, a single amino acid of the D- or L- configuration, or a di- or poly-peptide containing amino  
10 acids of the D- or L- configuration, or a combination of these,

A<sup>0</sup> is D-Arg or another basic or neutral aromatic, aliphatic, heterocyclic or alicyclic amino acid of the D- or L- configuration,

B<sup>1</sup> is Arg or another basic or neutral aromatic, aliphatic, heterocyclic or alicyclic amino acid of the D- or L- configuration,

15 C<sup>2</sup> is Pro, Hyp, or another basic or neutral aromatic, aliphatic, heterocyclic, or alicyclic amino acid of the D- or L- configuration,

D<sup>3</sup> is Pro, Hyp, or another basic or neutral aromatic, aliphatic, heterocyclic, or alicyclic amino acid of the D- or L- configuration,

E<sup>4</sup> is Gly, Niga, Nigb or another basic or neutral aromatic, aliphatic,  
20 heterocyclic, or alicyclic amino acid of the D- or L- configuration,

F<sup>5</sup> is Igla, Iglb, Niga, Nigb, Phe, Thi, Cpg, Chg, or another aliphatic, aliphatic heterocyclic, or alicyclic amino acid of the D- or L- configuration,

G<sup>6</sup> is Ser, Ser(SO<sub>4</sub>), HBQ or another aromatic, aliphatic, heterocyclic, or alicyclic amino acid of the D- or L- configuration,

25 H<sup>7</sup> is Igla, Iglb, Niga, Nigb, Tic, Cpg, Chg, or another aliphatic, aliphatic heterocyclic, or alicyclic amino acid of the D- or L- configuration,

J<sup>8</sup> is Igla, Iglb, Niga, Nigb, Tic, Nbn, Oic, Cpg, Chg, Leu or another aromatic, aliphatic, aliphatic heterocyclic, or alicyclic amino acid of the D- or L- configuration, and

30 Z represents the terminal carboxy group. Where Z is not set forth in the above formula, it is understood to be present.

According to the above formula, X and A may be alternatively described as being optionally absent in which case they may represent the terminal amino group. Where X is present, it is preferably a single amino acid residue or a di-peptide.

- 5        The bradykinin antagonist peptides of the present invention may be illustrated by the following (alignment of the residues in a particular row does not imply nor limit to a given peptide sequence):

	X --	A --	B --	C --	D --	E --	F --		G --	H --	J
10		0	1	2	3	4	5		6	7	8
	Aaa	DArg	Arg	Pro	Hyp	Gly	Igla		Ser	DIgla	Igla
	Aca	DLys	Lys	DMF	Pop	Iglb	Ser(SO <sub>4</sub> )		DIglb	Iglb	Lys
15	Acetyl	Arg	DArg	NMF	Niga	Nigb	Niga		HBQ	Niga	Niga
	Dhq	Lys	DLys	MPIV	MPIV	Ala	Nigb		Cys	Nigb	Nigb
	Nba	Gly	Cys	Hyp	Pro	Gly	Leu		Gly	DLeu	Leu
	Tba			Azt	Azt		Chg		DChg	Chg	NChg
	Cha			Dhp	Dhp		Ile		Dlle	Ile	
20	Cpa			Inip	Inip		Val		DVal	Val	
	Gun			Thz	Thz		Alg		DCpg	Cpg	
				Pop			Oic		DOic	Oic	
	Lys-Lys						Pop		DPop	Pop	
							Nle		DNle	Nle	
25							DMF		DDMF	DTic	
							Cpg		Tic		
							Thi		DTic		

In a preferred embodiment, the bradykinin antagonist peptides of the present invention are represented as follows:

30

- X is Gun or absent;  
 A<sup>0</sup> is DArg or Lys;  
 B<sup>1</sup> is Arg or Cys;  
 C<sup>2</sup> is Pro;  
 5 D<sup>3</sup> is Hyp;  
 E<sup>4</sup> is Gly;  
 F<sup>5</sup> is Igla, Iglb, Cpg or Thi;  
 G<sup>6</sup> is Ser;  
 H<sup>7</sup> is DIgla, DIglb, DCpg, Tic or DTic; and  
 10 J<sup>8</sup> is Leu, Cpg or Oic.

Preferred embodiments may be represented as follows:

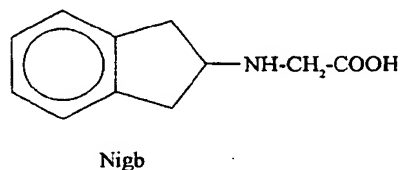
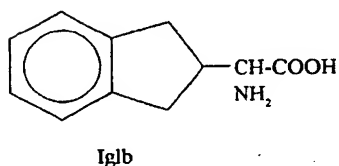
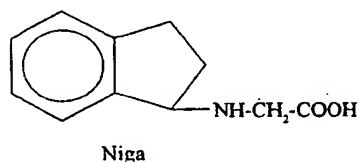
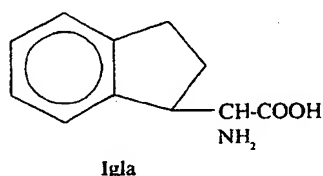
- |    |        |   |
|----|--------|---|
|    | SC-722 | DArg-Cys-Pro-Hyp-Gly-Cpg-Ser-DCpg-Cpg                     |
|    | SC-724 | DArg-Lys-Pro-Hyp-Gly-Cpg-Ser-DCpg-Cpg                     |
|    | B9004  | DArg-Arg-Pro-Hyp-Gly-Cpg-Ser-Tic-Cpg                      |
| 15 | B9006  | DArg-Arg-Pro-Hyp-Gly-Thi-Ser-Tic-Cpg                      |
|    | B9066  | DArg-Arg-Pro-Hyp-Gly-Cpg-Ser-DTic-Cpg                     |
|    | B9068  | DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Cpg                     |
|    | B9102  | DArg-Arg-Pro-Hyp-Gly-Cpg-Ser-DCpg-Cpg                     |
|    | B9810  | Gun-Gly-Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Leu (SEQ ID NO:1) |
| 20 | B9812  | DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic                     |
|    | B9814  | Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Leu                          |
|    | B9816  | DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Leu                     |
|    | B9822  | Gun- DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic                |
|    | B9824  | DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIgl-Oic                     |
| 25 | B9826  | Gun- DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIgl-Oic                |
|    | B9840  | DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DTic-Cpg                     |
|    | B9842  | Lys-Arg-Pro-Hyp-Gly-Igl-Ser-DTic-Cpg                      |
|    | B9856  | Lys-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic                      |
|    | B9858  | Lys- Lys-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic                 |
| 30 |        | DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-NChg                    |

More preferred embodiments of the present invention are:

Lys-Lys<sup>0</sup>-Arg<sup>1</sup>-Pro<sup>2</sup>-Hyp<sup>3</sup>-Gly<sup>4</sup>-Iglb<sup>5</sup>-Ser<sup>6</sup>-DIglb<sup>7</sup>-Oic<sup>8</sup>; and  
 Gun-DArg<sup>0</sup>-Arg<sup>1</sup>-Pro<sup>2</sup>-Hyp<sup>3</sup>-Gly<sup>4</sup>-Thi<sup>5</sup>-Ser<sup>6</sup>-DIglb<sup>7</sup>-Oic<sup>8</sup>.

5

According to this invention, the indane substituent can be on either the  $\alpha$ -carbon (residues abbreviated Igl) or the nitrogen (residues abbreviated Nig) of the glycine residue, and the indane residue can be attached to the glycine moiety at either position 1 (Igl or Nig) or position 2 (Iglb or Nigb) of the indane group.



As used herein, abbreviations of the natural amino acids are those accepted in the art (*Biochem. J.* 126: 773 (1972)), and unless prefixed with D are all of the L-configuration (except glycine and MPIV, which are not optically active).

Abbreviations used for unnatural amino acids in Bradykinin analogs are indicated below:

AC6	1-Aminocyclohexane-1-carboxylic acid
Alg	Allylglycine
20 Azt	Azetine-2-carboxylic acid (norproline)

	CDF	<i>p</i> -Chloro-D-Phe
	Chg	CyclohexylGly ( $\alpha$ -Aminocyclohexaneacetic acid)
	cLeu	1-Aminocyclopentane-1-carboxylic acid (cycloleucine)
	Cpg	CyclopentylGly ( $\alpha$ -Aminocyclopentaneacetic acid)
5	Dhp	3,4-Dehydro-Pro
	DMF	2,4-Dimethylphenylalanine
	Eac	6-Aminohexanoic acid ( $\epsilon$ -aminocaproic acid)
	FDF	<i>p</i> -Fluoro-DPhe
	Gun	Guanidyl
10	HBQ	N5-(4-hydroxybutyl)-glutamine
	Hig	Hexahydroindanylglycine
	Hyp	<i>trans</i> -4-Hydroxy-Pro
	Igla	$\alpha$ -(1-indanyl)glycine
	Iglb	$\alpha$ -(2-indanyl)glycine
15	Inip	Isonipecotic acid (piperidine-4-carboxylic acid)
	MDY	O-Methyl-DTyr
	MPIV	2,4-Methanoproline (2-Azabicyclo-(2,1,1)-hexane-1-carboxylic acid)
	Nal	$\beta$ -2-Naphthyl-Ala
20	Niga	N-(1-indanyl)glycine
	Nigb	N-(2-indanyl)glycine
	Nle	Norleucine
	NMF	N-Methylphenylalanine
	Oic	Octahydroindole-2-carboxylic acid
25	OMT	O-Methyl-Tyr
	Pal	$\beta$ -3-Pyridyl-Ala
	PCF	<i>p</i> -Chloro-Phe
	Pip	Pipecolic acid ("homo-Pro")
	Pop	<i>trans</i> -4-PropoxyPro
30	Ser(SO <sub>4</sub> )	Serine-O-sulfate
	Suc	Succinyl

Thi	$\beta$ -2-Thienyl-Ala
Thz	Thiazolidine-4-carboxylic acid
Tic	1,2,3,4-Tetrahydroisoquinoline-3-carboxylic acid

5 As used herein, the term "aromatic" shall mean aryl ( $C_6-C_{12}$ ), or heteroaryl, wherein the heteroaryl groups are mono- or bi-cyclic five or six membered rings containing one or more heteroatoms such as oxygen, sulfur or nitrogen in any combination and are optionally substituted with alkyl ( $C_1-C_4$ ), alkenyl ( $C_1-C_4$ ), halo, cyano, nitro, amino, hydroxy groups and the like.

10 As used herein, the term "aliphatic" shall mean alkyl ( $C_1-C_{12}$ ), alkenyl ( $C_1-C_{12}$ ) or alkynyl ( $C_1-C_{12}$ ) and heteroalkyl ( $C_5 - C_{12}$ ) or heteroalkenyl ( $C_5-C_{12}$ ).

As used herein, the term "alicyclic" shall mean optionally substituted cycloalkyl ( $C_4-C_{12}$ ), optionally containing 1-3 double bonds .

As used herein, the term "heterocyclic" shall mean mono- or bi-cyclic  
15 five or six membered rings containing one or more heteroatoms such as oxygen, sulfur or nitrogen in any combination, optionally substituted with alkyl ( $C_1-C_4$ ), alkenyl ( $C_1-C_4$ ), halo, cyano, nitro, amino, hydroxy groups and the like, and optionally containing 1 to 3 double bonds.

Abbreviations used for acylating groups (as used for "X") are as follows:

20

Aaa-	1-Adamantaneacetyl-
Ac-	Acetyl-
Aca-	1-Adamantanecarbonyl-
Bz-	Benzoyl-
25 Cha-	Cyclohexaneacetyl-
Cpa-	Cyclopentaneacetyl-
Dca-	2,2-Dicyclohexylacetyl-
Dhq-	2,3-Dehydroquinuclidine-3-carbonyl-
Dpa-	2,2-Diphenylacetyl-
30 Dpp-	3,3-Diphenylpropionyl-
Nba-	Norbornane-2-acetyl-

Nbc-	2-( <i>cis</i> -5-norbornene- <i>endo</i> -3-carbonyl)-
Nbi-	<i>cis</i> -5-norbornene- <i>endo</i> -2,3-dicarboximidyl-
Paa-	Phenylacetyl-
Pba-	4-Phenylbutyryl-
5 Ppa-	3-Phenylpropionyl-
Sin-	Sinapinyl- (3,5-dimethoxy-4-hydroxycinnamyl-)

The description of peptide synthesis methods uses several abbreviations for standard solvents, reagents and procedures, defined as follows:

10	BOP	Benzotriazolyl-oxy-tris-(dimethylamino)phosphonium hexafluorophosphate
	BuOH	n-Butanol
	DCC	Dicyclohexylcarbodiimide
15	DCM	Dichloromethane
	DIC	Diisopropylcarbodiimide
	DIEA	Diisopropylethyl amine
	DMF	Dimethylformamide
	HATU	O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
20	HOAc	Acetic acid
	MeOH	Methanol
	OHMR	Hydroxymethylpolystyrene resin for peptide synthesis, 1% crosslinked.
25	TBTU	O-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate
	TEA	Triethyl amine
	TFA	Trifluoroacetic acid

30 The following abbreviations for blocking groups used in synthesis are:

Boc	<i>t</i> -Butyloxycarbonyl
Tos	<i>p</i> -Toluenesulfonyl
Bzl	Benzyl ether

5 The following abbreviations for standard techniques used are:

AAA	Amino acid analysis (Stewart & Young p. 108)
CCD	Countercurrent distribution (Stewart & Young p. 96)
ELEC	Paper electrophoresis (Stewart & Young p. 117)
HPLC	High performance liquid chromatography (Stewart & 10 Young, p. 100)
Kaiser test	Ninhydrin test for completeness of coupling reactions (Stewart & Young, p. 105)
SPPS	Solid phase peptide synthesis
TLC	Thin-layer chromatography (Stewart & Young, p. 103)

15

The synthesis of peptides described herein, including preparation of appropriate amino acid derivatives, their activation and coupling to form peptides and methods for purification of peptides and determination of their purity are included in the general body of knowledge of peptide chemistry, as  
20 generally described in Houben-Weyl "Methoden der Organischen Chemie" Vol. 16, parts I & II, (1974) for solution-phase synthesis, and in "Solid Phase Peptide Synthesis" by Stewart and Young (1984) for synthesis by the solid phase method. A chemist skilled in the art of peptide synthesis would be able to synthesize the described peptides by standard solution methods or by manual or  
25 automatic solid phase methods.

Therapeutic application of bradykinin antagonists include treatment of traumatic, inflammatory or pathological conditions mediated by bradykinins or their closely related metabolites. These conditions may include treatment of bites, stings, general trauma, head trauma, inflammatory conditions including  
30 inflammatory bowel disease, burns, rashes, shock or hypotension associated with sepsis, and pain, especially pain associated with surgical or dental procedures. In

addition bradykinin antagonists may be used for the treatment of airway hypersensitivity and inflammation, as well as other symptoms associated with asthma. Bradykinin is recognized as a mitogenic agent and compounds disclosed in this invention have exhibited *in vitro* activity which may indicate  
5 their utility as anti-cancer agents. The compounds of the present invention may also be effective in the treatment of angiogenic diseases such as rheumatoid arthritis, diabetic retinopathy and cancer.

The compounds may be administered topically, or by injection or infusion or as an oral suspension in an appropriate vehicle or as tablets, pills,  
10 capsules, caplets or the like. The dosage and manner of administration will be defined by the application of the bradykinin antagonist and can be determined by routine methods of clinical testing to find the optimum dose. These doses are expected to be in the range of 0.001 mg/Kg to 100 mg/Kg of active compound.

The compounds are composed of amino acids which may form salts due  
15 to their acidic or basic nature, and any pharmacologically acceptable salt derived from the compounds described in this invention such as hydrochlorides, acetates, phosphates, maleates, citrates, benzoates, salicylates, succinates, ascorbates and the like, are considered an extension of this invention. A common tactic in medicinal chemistry is to modify known drug substances which are peptide  
20 based to form esters or amides which exhibit greater bioavailability. Prodrugs derived from the compounds disclosed here are therefore considered an obvious extension of this invention. Methods for designing and preparing prodrugs are described in detail in the medicinal chemical literature.

25

## EXAMPLES

### EXAMPLE I - Peptide Synthesis - General Methods

Synthesis of the bradykinin antagonist peptides of the present invention by solid phase peptide synthesis (SPPS) may be carried out manually (see Stewart & Young) or by use of the Beckman Model 990, Biosearch Model 9500  
30 or other automatic peptide synthesizers. SPPS involves use of certain standard procedures, defined as follows:

Procedure A: DCC coupling reaction: (Described in Stewart & Young, p 76ff). A 2.5-fold excess of Boc-amino acids over peptide-resin is used in the Model 990 synthesizer. Boc-amino acids are activated for coupling with an equimolar amount of DCC. The solvent may be DCM or mixtures of DCM and DMF. Completeness of coupling may be determined by use of the Kaiser reagent.

Procedure B: DIC coupling: In the Model 9500 synthesizer a 6-fold excess of Boc-amino acids over peptide-resin is used with an equimolar amount of DIC. The solvent is DCM:DMF (1:1). The resin is washed with the same solvent before and after coupling. Completeness of coupling is determined with the Kaiser reagent.

Procedure C: BOP, TBTU or HATU coupling reaction for hindered amino acids: A 3-fold excess of Boc-amino acid over peptide-resin is mixed with an equimolar amount of BOP, TBTU or HATU and 2 equivalents of DIEA in DMF. The peptide-resin is washed with DMF before and after the coupling reaction, and after coupling is then washed 2 times with methanol before continuing standard DCM washes. Completeness of coupling is checked by the Kaiser test. BOP, TBTU and HATU, in this order, show increasing ability to cause successful coupling of sterically hindered amino acids.

Procedure D: TFA deprotection and neutralization: (Stewart & Young p. 76). The deprotection reagent is TFA:DCM (1:3), containing 1 mg/ml indole. It is used for 30 minutes, following a prewash. The neutralization reagent is 10% TEA in DCM, prepared fresh and used twice for one minute.

Procedure E: Terminal deprotection: (Described by Stewart & Young, p. 79). Deprotection with TFA:DCM is carried out as described in Procedure D. The peptide-resin is then washed three times with DCM and three times with MeOH and dried.

Procedure F: HF cleavage and deblocking: (Stewart & Young p. 85). A batch of 500 mg (0.2 mmole) of peptide-resin is mixed with 1.0 ml anisole and chilled in the reaction vessel to -78° C and 10 ml of anhydrous HF is distilled into the vessel under vacuum. The mixture is stirred at 0° C for 45 min, and the

HF is evaporated under vacuum. The peptide and resin mixture is washed three times with dry ether, and the peptide is extracted into glacial HOAc. The peptide solution is lyophilized.

Procedure G: PURIFICATION OF PEPTIDES: (Stewart & Young p. 96). The peptides may be purified by CCD for 100 transfers in the appropriate system, as determined by preliminary *k* estimation. Examples of CCD systems are:

A: n-BuOH:1% TFA for average antagonist peptides

B: n-BuOH:ethyl acetate:1% TFA (1:1:2) for more hydrophobic antagonist peptides.

Procedure H: TLC: TLC may be carried out on silica gel plates with systems F (n-BuOH:HOAc:H<sub>2</sub>O:pyridine =15:3:8:10) and I (n-BuOH:HOAc:H<sub>2</sub>O=4:1:1). Chlorine-tolidine and Sakaguchi spray reagents may be used.

Procedure J: Paper electrophoresis (ELEC): ELEC may be done in buffers of pH 2.8 and 5.0 as described in Stewart & Young. Chlorine-tolidine and Sakaguchi spray reagents may be used.

Procedure K: HPLC: Preparative HPLC may be carried out on large-pore reversed phase C4 or C8 silica columns in a gradient of 0.1% TFA in H<sub>2</sub>O to 0.08% TFA in acetonitrile. Detection may be by UV at 214 or 235 nm. Analytical HPLC may be carried out in the same system and in a gradient of acetonitrile in 0.25M triethylammonium phosphate, pH 6.5.

Procedure L: MASS spectroscopy: Peptides may be checked for the correct molecular mass by fast atom bombardment (FAB) or laser desorption (MALDI) mass spectroscopy.

Procedure M: Amino acid analysis (AAA): Peptides may be hydrolyzed in 6N HCl and analyzed as described in Stewart & Young, pp 109-112, using a Beckman Model 6300 amino acid analyzer.

**EXAMPLE II - Synthesis of des-Arg<sup>9</sup>-BK antagonists****Esterification of N-Boc-protected unusual amino acids**

(cyclopentaneglycine or octahydro-indole-2-carboxylic acid) to the chloromethyl resin was achieved easily without racemization by means of their cesium salts

- 5 using the standard procedure (Stewart et al., Solid Phase Peptide Synthesis, 2nd ed. Pierce Chemical Company: Rockford, IL, 1984; pp. 85-88).

- A. Cesium Salts of Boc-amino Acids: A solution of Boc-protected unusual amino acid (Boc-cyclopentaneglycine or Boc-octa-hydroindole-2-carboxylic acid) (0.475 mmol) in methanol (20 ml) and water (2 ml) was titrated  
10 to pH 7 with 20% aqueous cesium carbonate. The solution was evaporated to dryness in vacuo. The residual protected amino acid cesium salt was dried by addition of DMF and re-evaporation two or three times at 45 C. They were dried over P205 for 5 h.

**B. Attachment of Boc-amino acid cesium salts to chloromethyl resin:**

- 15 0.44 mmol of dry Boc-cyclopentaneglycine or Boc-octahydroindole-2-carboxylic acid cesium salt and 1 g (0.4 mmol) of chloromethyl resin in anhydrous DMF (15 ml) were stirred at 50°C for 15-24 h. The resin was filtered and washed with DMF, DMF: H<sub>2</sub>O (1:1), DMF, MeOH and DCM by the standard procedure.

- The desired peptide sequences were assembled on the above amino acid-  
20 resins using standard procedures, to give potent [des-arg<sup>9</sup>]-bradykinin analogs

BSH = 3D Bissuccinimidohexane

Mosi = 3D Methoxy-suberimido

Sub = 3D Suberyl

- 25 Suc = 3D Succinyl

Suim = 3D Suberimidyl

**EXAMPLE III - Synthesis of  $\alpha$ -(2-indane)-glycine (Iglb)**

- 2-Bromoindane: To 2-indanol (Aldrich) (105 g, 0.78 mol) in pyridine  
30 (16 mL, 0.2 mol) and 340 mL of chloroform at -15°C was added PBr<sub>3</sub> (84 mL, 0.89 mol) over 45 min. The reaction mixture was stirred overnight at room

temperature and extracted by addition of 450 mL of chloroform and 500 g of ice. The organic layer was washed twice with water, and dried over  $\text{Na}_2\text{SO}_4$ . The solvent was evaporated in vacuo, leaving a brown semi-solid. The product was distilled rapidly in vacuo and then fractionated to give 69g (45%) 2-

5 bromoindane, bp 90-93°/3 mmHg;  $n_D^{23} = 1.5837$ .

Ethyl  $\alpha$ -acetamido- $\alpha$ -cyano-2-indaneacetate: To sodium ethoxide (20.4 g, 0.3 mol), suspended in dry DMSO (250 mL) was added a solution of ethyl acetamidocyanoacetate (50 g, 0.294 mol) in 250 mL dry DMSO, with vigorous stirring. Then 2-bromoindane (65.0 g, 0.33 mol) was added dropwise  
10 during 40 min, with vigorous stirring. The brown solution was stirred overnight at room temperature and 4 h at 50°C. The mixture was evaporated in vacuo and the residue was treated with 300 mL cold water and extracted twice with 250 mL of EtOAc. The combined extracts were dried ( $\text{MgSO}_4$ ) and evaporated to give the crude product, 70.7 g brown solid. The first recrystallization from EtOH/ $\text{H}_2\text{O}$   
15 gave 58.6 g yellowish solid, mp 153-157°. The second recrystallization from toluene gave 54.6 g (64.9%) white flakes, mp 159-161°C.

D,L-2-indaneglycine: A solution of 54.6 g (0.19 mol) ethyl  $\alpha$ -acetamido- $\alpha$ -cyano-2-indaneacetate in 820 mL of 10% NaOH was refluxed for 20 h. The solution was cooled, decolorized with carbon, and the filtrate, in an ice bath, was  
20 adjusted to pH 6.5 with conc. HCl (about 150 mL), using a pH meter, and further chilled to complete precipitation of the product, which was filtered and washed with cold water, methanol and ether; 36.4 g. The solid was refluxed in 1200 mL 6N HCl for 10 h; the solution was decolorized with carbon and chilled to give a precipitate, which was collected and recrystallized from  $\text{H}_2\text{O}$  to give 22.6 g  
25 product. Additional product was obtained from the mother liquor to give 31.7 g (86.9%) of D,L-2-indaneglycine.

N-acetyl-D,L-2-indaneglycine: To a mixture of 6.83 g (0.03 mol) of D,L-2-indaneglycine and 54 mL  $\text{H}_2\text{O}$  was added 30 mL (0.06 mol) 2N NaOH. The solution was chilled and stirred while 1.4 mL (0.015 mol) acetic anhydride  
30 was added. Seven successive additions of 2N NaOH (14 mL) and 1.4 mL of acetic anhydride were done over 30 min, and the solution was allowed to warm

to room temperature, with continued stirring. Stirring was continued overnight. The solution was chilled and acidified to pH 3 with 6N H<sub>2</sub>SO<sub>4</sub> (40 mL). After standing in the cold, the precipitate was collected and washed with a small amount of cold H<sub>2</sub>O; 7.95 g, mp 199-202°. The product was recrystallized from  
5 acetone/petroleum ether (30-60°) to give 7.8 g acetyl D,L-2-indaneglycine, mp 201-203°.

Resolution of D,L-2-indaneglycine: N-acetyl-D,L-2-indaneglycine (7.0 g, 0.03 mol) was suspended in 300 mL H<sub>2</sub>O, and the pH was adjusted to 7.6 with 4N LiOH (9 mL). Water was added to a volume of 350 mL, the solution  
10 was thermostatted at 37°C, with stirring, and hog kidney acylase I (Sigma A-3010, 50 mg) was added. The pH was maintained at 7.6 by addition of LiOH; after precipitation of L-indaneglycine began, the pH rose and was brought back with 0.1 N HOAc. After 5 h an additional 30 mg acylase I was added; and after 24 h an additional 20 mg acylase was added, with continued pH control.  
15 Incubation, with stirring was for a total of 36 h. The solution was cooled to room temperature, and 250 mL cold water and 500 mL EtOAc were added. The mixture was carefully acidified to pH 0.75 with 6 N HCl (about 25 mL). The two layers were wet filtered through Celite and separated. The water phase was extracted twice with EtOAc (250 mL).

20 The aqueous solution was decolorized with carbon at 50° and evaporated under reduced pressure. The crystals were dissolved in 25 mL H<sub>2</sub>O and 25 mL 6 N HCl and the cold solution was brought to pH 5.5 with conc. NH<sub>4</sub>OH. The product was collected, washed with cold water, and dried; 2.8 g (97.6%) L-2-indaneglycine; mp 302-305°;  $[\alpha]_D^{25} = +35.4^\circ$  (c 2, 2N HCl).

25 The ethyl acetate phase was washed with saturated NaCl solution, dried over MgSO<sub>4</sub> and evaporated in vacuo. The solid residue was recrystallized from EtOH/petroleum ether to give 3.1 g (88.6%) acetyl-D-2-indaneglycine, mp 210-212°;  $[\alpha]_D^{25} = -38.7^\circ$  (c 2, EtOH).

30 Acetyl D-2-indaneglycine (2.92 g, 0.0125 mol) was refluxed in 125 mL of 6 N HCl for 8 h. The solution was evaporated under reduced pressure at 40°, and the residue was dissolved in 40 mL 6 N HCl and 150 mL H<sub>2</sub>O. The solution

was neutralized to pH 5.5 with conc.  $\text{NH}_4\text{OH}$ , and the white solid was collected, washed with cold water and dried in vacuo to yield 2.17 g (90.8%) of D-2-indaneglycine; mp 302-305°;  $[\alpha]_D^{23} = -34.6^\circ$  (c 2, 2N HCl).

Both the D- and L-isomers were converted to the N-Boc derivative by the  
5 standard procedure (see the following Example).

**EXAMPLE IV - Synthesis of N-Boc-N-(2-indanyl) glycine(Boc-Nigb)**

Synthesis of N-(2-indanyl)-glycine (Nigb): Glycine methyl ester (3.49 g, 0.025 mole) and 2-indanone (4.96 g, 0.0375 mole) were dissolved in EtOH and  
10 then  $\text{NaCNBH}_3$  (4.71g, 0.075 mole) was added portionwise during about 30 min. The mixture was stirred at room temperature for 24 h. The EtOH was removed under reduced pressure and the residue was treated with water. The product was extracted by several extractions with EtOAc. The organic phase was washed with saturated aqueous  $\text{NaHCO}_3$ , dried over  $\text{Na}_2\text{SO}_4$  and evaporated. The product  
15 was purified by chromatography over silica gel (EtOAc and EtOAc/MeOH = 9:1) to give the crude ester as a colorless oil (2.46 g). The ester (2.43 g in 30 mL MeOH) was saponified with 15 mL 1N NaOH at room temperature for 5 h. The solution was concentrated and the residue was taken up in dioxane-water.

Synthesis of the Boc-derivative: The solution was cooled to 0° and  
20 treated with Boc anhydride (3.27 g, 0.015 mole) portionwise. The ice bath was removed, and the mixture was stirred overnight at room temperature, with adjustment of pH to 9.0 with NaOH solution as needed. The solution was evaporated to dryness under reduced pressure, the residue was taken up in EtOAc/water (70/30 mL), and the solution was treated with saturated aqueous  
25 citric acid solution to pH 2.5. The phases were separated and the water was extracted twice with EtOAc. The organic phase was washed with water, saturated NaCl solution, dried over  $\text{Na}_2\text{SO}_4$  and evaporated under reduced pressure. The crude product was recrystallized from EtOAc/hexanes; 1.85 g (25.4%), mp 130-131° C.

**EXAMPLE V -Binding Assays**

Human lung fibroblasts IMR-90 cells were obtained from ATCC and propagated in DMEM media in 850 mm roller bottles until confluent. Three hours prior to harvesting, the cells were treated with Interleukin 1b (200 pg/ml).

- 5 Human BK2 clones were propagated in F12 media until confluent. Preparation of membranes for binding assays was carried out by scraping cells from roller bottles in ice cold PBS and centrifuging at 1000 xg, at 4°C for 15 minutes. The supernatant was discarded and pellet resuspended in Buffer A consisting of 25 mM TES (pH 6.8) with 2 uM 1,10-Phenanthroline, and centrifuged at 27,000
- 10 xg for 15 min. this was then repeated. The final pellet was resuspended in Buffer B (Buffer A with 2 uM Captopril, 140 ug/ml Bacitracin, 0.1%BSA), and stored in 1 ml aliquots, frozen at -20°C until needed.

- Binding assays were performed by incubating human clone membranes with 0.3 nM <sup>3</sup>H-Bradykinin or IMR-90 membranes with 0.5 nM <sup>3</sup>H-des-Arg<sup>9</sup>-
- 15 Kallidin in the presence of the peptides in assay buffer (Buffer B with 1 mM Dithiotreitol), at room temperature, for 45 minutes. All test compound dilutions were in triplicate. Assays were harvested by quick filtration in a Tomtec Harvester 96, with ice cold wash buffer consisting of 10 mM Tris/HCl, pH 7.5, 100 mM NaCl, .02%BSA, onto Wallec printed glassfiber Filtermat "B", which
- 20 had been pre-soaked with 0.1% PEI and previously air-dried. Filtermats were counted in 9.5 mls Wallec Beta-Plate Scint, in Wallec 1450 MicroBeta Counter.

Table I - Binding Data

Bradykinin Antagonists (desArg <sup>9</sup> )	Human Binding - pIC <sub>50</sub>		GPI Binding - pIC <sub>50</sub>
	B1	B2	B2
B9004(17-27)	8.5	5.8	5.2
B9006(13-23)	7.9	6.2	5.0
B9066(74-86)	9.3	5.9	5.6
B9068(74-84)	8.8	5.7	5.1
B9810(15-27)	8.9	i.a	i.a
B9812(HPLC)	9.5	7.6	6.5
B9814(HPLC)	8.2	i.a	i.a
B9816(HPLC)	8.9	6.2	5.7
B9822(HPLC)	9.0	7.1	6
B9824(HPLC)	9.6	7.8	6.7
B9826(HPLC)	9.9	8.1	7.1
B9840(HPLC)	9.1	6.2	i.a
B9842(HPLC)	9.6	6.2	i.a.
B9856(HPLC)	9.6	7.6	6.3
B9858(HPLC)	10.1	7.7	6.9

i.a. = inactive. pIC<sub>50</sub> = -log of the IC<sub>50</sub> (concentration in molar which inhibits tritiated ligand binding by 50%).

## 25 EXAMPLE VI - In Vivo

Mongrel dogs of either sex, weight range 10-15 kg were used. These were anaesthetized with pentobarbitone, 30 mg/kg i.v. and catheters placed in one femoral artery and both femoral veins for the recording of blood pressure and infusion and injection of compounds. Responses to (50 nM) were produced in the absence and presence of antagonist and the ED<sub>50</sub> calculated. After the end of the infusion of the highest dose of the antagonist compound, BK and des-Arg<sup>9</sup>-BK were injected at intervals for up to four hours.

Table II - *In vivo* B2/B1 Data

		BK1 ED <sub>50</sub> μg/kg/min	Selectivity (BK, desArg9BK, ACh, NE, Sp, AII)	Duration of Action (following 20 min of highest dose)
CP-0298	desArg9 Lys0 Leu8 BX	0.546 ± .16 (558 pmol)	BK1 at 3.0 μg/kg/min	Almost 100% returned by 15-30 min
B9812		0.02 ± 0.003 (10.7 pmol)	B1 only at 1.0	B1 still 100% blocked at 4.6 h
B9858		0.03 ± 0.01	B1 only at 1.0	1.0 μg/kg/min ≥ 50% returned at 4.5 h 0.3 μg/kg/min 100% returned at 3.5-4 h

ED<sub>50</sub> = dose of antagonist to inhibit hypotensive response to desArg9BK by 50%.

**EXAMPLE VII - Rat Kidney Brush Border Microvilli**

Kidney membranes were prepared as indicated in the literature (Booth et al., *Biochemical Journal*, 142: 575 (1974)). Rat kidneys were obtained after sacrifice and rinsed in several volumes of a 2 mM tris, 10 mM mannitol buffer, pH 7.1. Cortical material was carefully removed and homogenized in ten volumes of the buffer. The homogenate was spun in a IEC Centra-8 refrigerated centrifuge. (4°C, 200 xg). The supernatant was made 10 mM with respect to  $\text{MgCl}_2$ . The supernatant material was subjected to further centrifugation as indicated:

10

supernatant

↓

(12 min, 1500 xg)

15

supernatant

↓

(12 min, 15,000 xg)

↓

Pellet. The pinkish layer on top of pellet was removed and resuspended  
20 in 100 ml mannitol-tris buffer made 10 mM  $\text{MgCl}_2$

↓

(12 min. 2200 xg)

↓

Supernatant

25

↓

(12 min, 15,000 xg)

↓

Pellet resuspended in tris-mannitol buffer. Protein concentration was determined  
by the method of Bradford (*Anal. Biochem.*, 72:248-254 (1976)).

30

**EXAMPLE IX - Rat, Human Lung Membrane**

Rat or human lung (3 gm) was prepared as a modification of Skidgel et al. (*Biochemical Pharmacology* 33: 3471 (1984)). Tissues were removed and placed in cold phosphate-saline (3x). Material was minced and homogenized in 20 volumes (w/v) of 0.5 mM tris HCl. 50 mM mannitol, pH 7.0. Calcium chloride was added to the homogenate to give a final concentration of 10 mM. The material was filtered through a gauze pad to remove loose connective tissue. Filtrate was spun in a Beckman centrifuge (3,000 xg) for thirty minutes. The resultant supernatant fraction was spun in a Beckman L7-65 ultracentrifuge (43,000 xg, 30 min) to spin down membranes. The centrifugation step was repeated on the membrane pellet fraction two additional times. The pellet was reconstituted in 4 ml of the tris mannitol buffer and stored at -70°C. Protein was determined by the method of Bradford.

**EXAMPLE IX - Stability**

Bradykinin antagonists were prepared at 1 mM concentration in a phosphate-buffered saline solution (phosphate saline, 2.6 mM  $\text{KH}_2\text{PO}_4$ , 10.42 mM  $\text{Na}_2\text{HPO}_4$ , 0.1454 M NaCl, pH. 7.2). Aliquots (10  $\mu\text{l}$ ) of the compound were added to 90  $\mu\text{l}$  of the tissue samples in Eppendorf tubes. Samples were incubated various time points at 37°C. At each time point protein was precipitated by addition of acetonitrile, acetonitrile made 0.1 M HCl or 10% (v/v) trifluoroacetic acid in water (100  $\mu\text{l}$ ). Tubes were spun (14,000 rpm, 10 min) in an Eppendorf Model 5415C microcentrifuge. Samples were filtered and analyzed using reverse-phase HPLC ( $\text{C}_{18}$  column 4.6 x 150 mm) and a 13.5% to 90% acetonitrile gradient in 0.1% TFA. Compound half-life was determined by analysis of raw data using ENZFIT application software (Elsevier).

<sup>1</sup>HPLC: Waters Model 712 Autosampler, Model 484 Variable Wavelength Detector, and Millipore Model 810 Application software.

TABLE III  
Stability of Compounds CP-0298,  
B9812 & B9066 in Biological Fluids

5	Compound	Half-Life (h)
	(Rat kidney Brush Border)	
	Bradykinin	0.13
	CP-0298	0.020
	B9066	>> 6
10	B9812	>> 6
	(Rat Lung Membranes)	
	Bradykinin	0.13
	CP-0298	0.085
	B9066	>> 6
15	B9812	>> 6

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

## (i) APPLICANT:

- (A) NAME: SCHWEGMAN, LUNDBERG, WOESSNER & KLUTH, P.A.
- (B) STREET: 3500 IDS CENTER
- (C) CITY: MINNEAPOLIS
- (D) STATE: MINNESOTA
- (E) COUNTRY: USA
- (F) POSTAL CODE (ZIP): 55402
- (G) TELEPHONE: 612-339-0331
- (H) TELEFAX: 612-339-3061

(ii) TITLE OF INVENTION: DES-ARG9-BK ANTAGONISTS

(iii) NUMBER OF SEQUENCES: 1

## (iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(EPO)

## (v) CURRENT APPLICATION DATA:

APPLICATION NUMBER: Unknown

## (2) INFORMATION FOR SEQ ID NO: 1:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 11 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Xaa Gly Lys Arg Pro Pro Gly Phe Ser Pro Leu  
1                      5                      10

## CLAIMS

We claim:

1. A bradykinin antagonist of the formula:

5 X--A<sup>0</sup>--B<sup>1</sup>--C<sup>2</sup>--D<sup>3</sup>--E<sup>4</sup>--F<sup>5</sup>--G<sup>6</sup>--H<sup>7</sup>--J<sup>8</sup>--Z.

wherein

X is absent or is an aromatic, aliphatic, aromatic-aliphatic, alicyclic, heterocyclic or urethane-type acylating group, or at least one amino acid;

$A^0$ ,  $B^1$ ,  $C^2$ ,  $D^3$ , and  $E^4$  are basic or neutral aromatic, aliphatic,

10 heterocyclic, or alicyclic amino acids or A<sup>0</sup> is absent;

G<sup>6</sup> is an aromatic, aliphatic, heterocyclic, or alicyclic amino acid;

F<sup>5</sup>, H<sup>7</sup> and J<sup>8</sup> are aromatic, aliphatic, aliphatic heterocyclic, or alicyclic amino acids, provided that at least one of F, H and J is selected from Cpg, Cpgg, Igl<sub>a</sub>, Igl<sub>b</sub>, Nig<sub>a</sub> and Nig<sub>b</sub> of the D- or L- configuration, and

15            Z is COOH.

2. The bradykinin antagonist peptide according to claim 1, wherein

$A^0$  and  $B^1$  are basic amino acids:

$C^2$  is Pro;

20                     $D^3$  is Hyp;

E<sup>4</sup> is Gly;

F<sup>5</sup> is an Indanyl amino acid;

**G<sup>6</sup> is Ser;**

H<sup>7</sup> is a D-Indanyl amino acid; and

25 J<sup>8</sup> is an Indanyl amino acid.

3. The bradykinin antagonist according to claim 1, wherein

X is Aaa, Aca, Acetyl, Dhq, Nba, Tba, Cha, Gun or Cpa;

$A^0$  is DArg, DLys, Arg or Lys;

30 B' is DArg, DLys, Arg, Cys or Lys;

C<sup>2</sup> is Pro, DMF, NMF, MPIV, Hyp, Azt, Dhp, Inip, Thz, or Pop;

D<sup>3</sup> is Hyp, Pop, Niga, MPIV, Pro, Azt, Dhp, Inip, or Thz;

E<sup>4</sup> is Iglb, Nigb, Ala, or Gly;

F<sup>5</sup> is Igla, Ser(SO<sub>4</sub>), Niga, Nigb, Leu, Chg, Ile, Val, Alg, Oic, Pop, Nle,

Cpg, Thi or DMF;

5 G<sup>6</sup> is Ser, DIglb, HBQ, Cys, or Gly

H<sup>7</sup> is DIgla, Iglb, Niga, Nigb, DLeu, DChg, DIle, DVal, DCpg, DOic,

DPop, DNle, Tic, DTic or DDMF; and

J<sup>8</sup> is Igla, Lys, Niga, Nigb, Leu, Chg, Ile, Val, Cpg, Oic, Pop, or Nle.

10 4. A bradykinin antagonist according to claim 3 wherein

X is Gun or absent;

A<sup>0</sup> is DArg or Lys;

B<sup>1</sup> is Arg or Cys;

C<sup>2</sup> is Pro;

15 D<sup>3</sup> is Hyp;

E<sup>4</sup> is Gly;

F<sup>5</sup> is Igla, Iglb, Cpg or Thi;

G<sup>6</sup> is Ser;

H<sup>7</sup> is DIgla, DIglb, DCpg, Tic or DTic; and

20 J<sup>8</sup> is Leu, Cpg or Oic.

5. The bradykinin antagonist according to claim 4, selected from

DArg-Cys-Pro-Hyp-Gly-Cpg-Ser-DCpg-Cpg;

DArg-Lys-Pro-Hyp-Gly-Cpg-Ser-DCpg-Cpg;

25 DArg-Arg-Pro-Hyp-Gly-Cpg-Ser-Tic-Cpg;

DArg-Arg-Pro-Hyp-Gly-Thi-Ser-Tic-Cpg;

DArg-Arg-Pro-Hyp-Gly-Cpg-Ser-DTic-Cpg;

DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Cpg;

DArg-Arg-Pro-Hyp-Gly-Cpg-Ser-DCpg-Cpg;

30 Gun-Gly-Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Leu (SEQ ID NO:1);

DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic;

Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Leu;  
DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Leu;  
Gun- DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic;  
DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIgl-Oic;  
5 Gun- DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIgl-Oic;  
DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DTic-Cpg;  
Lys-Arg-Pro-Hyp-Gly-Igl-Ser-DTic-Cpg;  
Lys-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic; and  
Lys- Lys-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic.

10

6. The bradykinin antagonist according to claim 1, selected from  
Lys-Lys<sup>0</sup>-Arg<sup>1</sup>-Pro<sup>2</sup>-Hyp<sup>3</sup>-Gly<sup>4</sup>-Iglb<sup>5</sup>-Ser<sup>6</sup>-DIglb<sup>7</sup>-Oic<sup>8</sup>; and  
Gun-DArg<sup>0</sup>-Arg<sup>1</sup>-Pro<sup>2</sup>-Hyp<sup>3</sup>-Gly<sup>4</sup>-Thi<sup>5</sup>-Ser<sup>6</sup>-DIglb<sup>7</sup>-Oic<sup>8</sup>.

15 7. A method of treating acute or chronic inflammation comprising  
administering to a mammalian host in need of such treatment a therapeutic  
amount of the bradykinin antagonist according to claim 1.

8. A method of treating angiogenic diseases comprising administering to a  
20 mammalian host in need of such treatment a therapeutic amount of the  
bradykinin antagonist according to claim 1.

9. The method according to claim 8 wherein the angiogenic disease is  
rheumatoid arthritis.

25

10. The method according to claim 8 wherein the angiogenic disease is diabetic  
retinopathy.

11. The method according to claim 8 wherein the angiogenic disease is cancer.

# INTERNATIONAL SEARCH REPORT

Int. .onal Application No  
PCT/US 96/13614

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 C07K7/18 A61K38/08

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,0	KININ '95, 14TH INT. SYMP. BRADYKININ AND RELATED KININS, 1995, XP002020853 & IMMUNOPHARMACOLOGY, vol. 33, 1996, pages 183-185, XP000610704 GERA, L. ET AL.: "New bradykinin antagonists having very high potency at B1 receptors" * whole disclosure * --- -/--	1-11

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents :

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- \*&\* document member of the same patent family

Date of the actual completion of the international search

11 December 1996

Date of mailing of the international search report

21.01.97

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+ 31-70) 340-3016

Authorized officer

Hermann, R

# INTERNATIONAL SEARCH REPORT

Int ional Application No  
PCT/US 96/13614

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,0	KININ '95 , 14TH INT. SYMP...., 1995, XP002020854 & IMMUNOPHARMACOLOGY, vol. 33, 1996, pages 51-60, XP000610702 STEWART, J.M. ET AL.: "A new generation of bradykinin antagonists" * whole disclosure *	1-11
Y	BR. J. PHARMACOL., vol. 109, 1993, pages 14-17, XP000612729 HU, D-E. & FAN, T-P. D.: "[Leu8]des-Arg9-bradykinin inhibits the angiogenic effect of bradykinin and interleukin-1 in rats" cited in the application * abstract *	1-11
Y	BIOPOLYMERS, vol. 33, no. 8, 1993, pages 1237-1247, XP002020829 LIU, X. ET AL. : "Proton magnetic resonance studies of bradykinin antagonists" * table 1; discussion *	1-11
A	EP,A,0 370 453 (HOECHST AG) 30 May 1990	

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 96/13614

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-370453	30-05-90	AT-T- 107318	15-07-94
		AU-B- 612054	27-06-91
		AU-A- 3943189	31-05-90
		DE-A- 3938751	31-05-90
		DE-D- 58907889	21-07-94
		ES-T- 2057071	16-10-94
		FI-B- 94353	15-05-95
		HU-B- 210566	29-05-95
		HU-A- 9500069	28-04-95
		IE-B- 63490	03-05-95
		IL-A- 91298	30-05-94
		JP-A- 2184697	19-07-90
		JP-B- 7121956	25-12-95
		LT-A,B 717	31-01-95
		LV-B- 10720	20-02-96
		NO-B- 177597	10-07-95
		PT-B- 91692	31-05-95